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# Systemic Endotoxaemia and Fibrinolysis During Aortic Surgery

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***Objective:*** *To determine whether endotoxaemia and activation of the systemic fibrinolytic system occurs during and after aortic surgery.*
***Design:*** *Prospective clinical study.*
***Setting:*** *University Hospital.*
***Materials:*** *31 patients undergoing aortic surgery.*
***Chief Outcome Measures:*** *Venous blood assay for endotoxin and plasminogen activator inhibitor-1 (PAI-1). Samples were obtained preoperatively, immediately before and 5 minutes after cross-clamp application and removal, and at 2, 4, 6 and 24 hours postoperatively. Tonometric sigmoid intramural pH was monitored throughout this period as a means of detecting colonic mucosal ischaemia.*
***Main Results:*** *Endotoxin levels increased after clamping of the aorta, peaking immediately before clamp removal, mean value 34.5 pg/ml,* $p < 0.01$*, but returning to preoperative levels by 24 hours. PAI-1 levels progressively increased after surgery, with persistently high levels remaining at 24 hours* ($p < 0.01$).
***Conclusions:*** *Endotoxaemia does occur during aortic surgery and appears to be associated with activation of the systemic fibrinolytic system.*

*Key Words: Endotoxin; Colonic ischaemia; Aortic surgery; Plasminogen activator inhibitor-1.*

## Introduction

Septicaemic shock is a serious clinical condition with a high mortality and is frequently accompanied by activation of the coagulation system, leading to consumption of coagulation factors and fibrinolytic activation.[1] Death in irreversible shock is due to multiple system organ failure caused in part by thrombotic occlusions of the microvasculature, and fibrinolytic enzymes and their inhibitors may be intimately involved in this process.[2] Plasminogen activator inhibitor-1 (PAI–1) is a specific inhibitor of plasminogen activators normally found in plasma, platelets and endothelial cells.

Multiple system organ failure (MSOF) is a recognised but fortunately uncommon complication of aortic surgery, and may occur as a result of colonic ischaemia.[3] The latter is reported to occur in 2–29% of patients undergoing aortic reconstruction,[4,5] and is more likely after surgery for aneurysm, particularly if ruptured.[6,7] Many methods of intraoperative monitoring for ischaemia are now employed routinely: measurement of inferior mesenteric arterial (IMA) stump pressure,[4] photoplethysmography,[8] intravenous fluorescein,[9] Doppler ultrasound,[10] laser Doppler flowmetry,[11] and measurement of sigmoid intramural pH.[12] The latter technique, pioneered by Fiddian-Green *et al.*, showed that transient sigmoid ischaemia was associated with disruption of the mucosal barrier to enteric microorganisms,[3] and suggested that bacterial translocation in such patients may cause MSOF and death. Increased intestinal permeability resulting from cross-clamp induced ischaemia has also been reported by others.[13] An important component of Gram-negative bacteria is the outer lipopolysaccharide membrane, fragments of which are known as endotoxins, and it is these endotoxins which are thought to be responsible for the clinical condition of Gram-negative sepsis.[14]

The aim of this prospective study was to determine whether endotoxaemia and fibrinolytic activation occurred in patients undergoing elective aortic surgery, and if so whether there was an association with colonic ischaemia.

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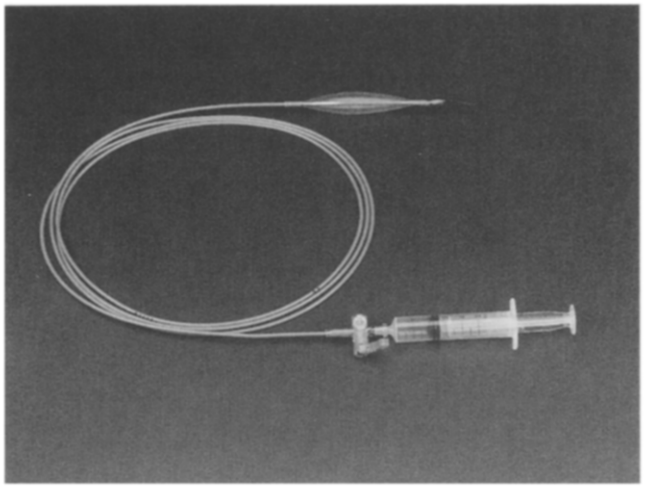

Fig. 1. The Sigmoid Tonometer.

## Materials and Methods

Thirty-one consecutive patients undergoing elective infrarenal aortic surgery, 18 for aneurysm and 13 for occlusive disease, were studied. A sigmoid tonometer, demonstrated in Fig. 1 (Tonometrics, Inc., Worcester, MA 01605, USA) was inserted per rectum into the sigmoid colon and the balloon filled with 2.5ml of normal saline; its position was checked at laparotomy. All operations were performed by the same surgeon using a standard technique of aortic graft insertion, the IMA being ligated in all aneurysm patients but none of those with occlusive disease, nor was reimplantation performed. Intra-operative fluid administration was governed by anaesthetic staff, losses being replaced by colloid, crystalloid and blood as required.

Samples were taken from the tonometer as follows: 1ml of saline was aspirated and discarded, then the remaining 1.5mls aspirated for $pCO_2$ measurement. The tonometer was then re-primed with a further 2.5mls of normal saline. These samples were taken immediately prior to cross-clamp application and removal, and at 2, 4, 6, and 24 hours after clamp application. Simultaneously arterial blood was taken for bicarbonate estimation; the intramural pH ($pH_i$) was then calculated using a modified Henderson-Hasselbach equation as follows:

$$pH^i = 6.1 + \log_{10}\left\{\frac{[HCO_3-]}{pCO_2\ (ss) \times 0.03}\right\}$$

where $pCO_2(ss)$ = measured $pCO_2$ × correction factor dependent on equilibration time since previous specimen.

Venous blood was taken for endotoxin assay at the above time points, with additional samples being taken preoperatively, 5 minutes after cross-clamp application and 5 minutes after its removal. Intra-operative specimens were taken directly from the inferior vena cava, later specimens from peripheral arm veins. Strict aseptic precautions were observed in order to prevent inadvertent contamination, each sample being taken via a separate venopuncture and emptied into special pyrogen-free tubes (Chromogenix, Molndal, Sweden). Specimens were put on ice and immediately transferred to the laboratory where they were centrifuged at 1200 revolutions per minute for 10 minutes in order to obtain platelet-rich plasma. This was frozen at –80°C and samples were later assayed in batches using the chromogenic COATEST limulus amoebocyte lysate (LAL) assay (Chromogenix).

Arterial and venous blood was taken at the same time points for measurement of PAI-1 activity. These samples were again immediately put on ice for transfer to the laboratory, where they were centrifuged at 3000 revolutions per minute for 15 minutes, the plasma obtained being frozen at –80°C for later batch assay. PAI-1 activity was measured in activity units/millilitre (AU/ml) as the plasma inhibition of single chain human t-PA[15] with the COATEST PAI enzyme-linked immunosorbent assay (Chromogenix, Molndal, Sweden).

Routine postoperative management included intravenous fluids, analgesia using intravenous morphine via a patient-controlled pump, and monitoring of pulse, blood pressure, central venous pressure, urine output, and peripheral pulses. Complications

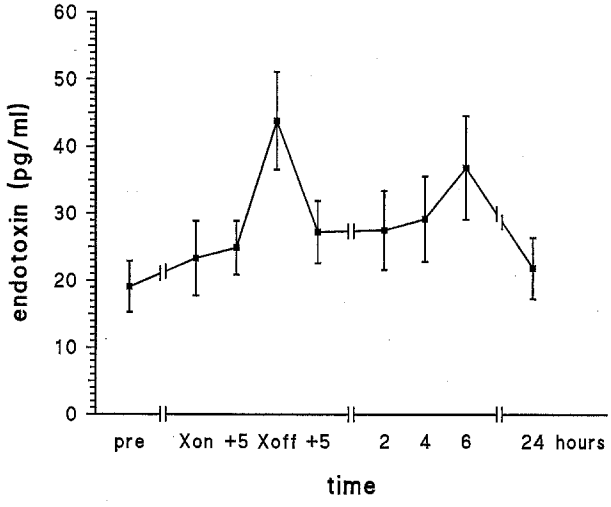


Fig. 2. Endotoxin results. Xon = cross-clamp on; Xoff = cross-clamp off.

were documented and treated appropriately, with particular attention to gastro-intestinal symptoms.

## Results

Endotoxin results measured in picogrammes per millilitre are presented in Fig. 2, overall mean values ± standard error of the mean from the 31 patients being plotted. It is clear that after cross-clamp application there is a marked increase in mean endotoxin value, peaking at a point immediately before clamp removal, with a second lesser peak after 6 hours. There were no significant associations between the development of endotoxaemia and age, sex, or cross-clamp time.

Venous and arterial PAI-1 results are similarly represented in Activity units per millilitre in Fig. 3. There is minimal activity during the cross-clamp period, but after clamp release there is a progressive rise.

Sigmoid intramural pH is demonstrated in Fig. 4. The dramatic fall seen after clamp application, mean $pH_i$ 6.98, recovers only gradually after its removal, with a 53% incidence of intramural acidosis after 6 hours, and 32% after 24 hours.

## Discussion

Aortic cross clamping is mandatory during graft placement, but such temporary vascular occlusion may result in ischaemic injury to the large intestine. Whilst clinically significant ischaemic damage is rare, nevertheless the reported overall incidence is 2–29%.[4,5] Ischaemia is more likely after surgery for ruptured aneurysm and ligation of a patent IMA is also a reported predisposing factor particularly if below the critical stump pressure of 40 mmHg,[4] though sigmoid mucosal $pH_i$, measured with the tonometer, has been shown superior in predicting ischaemic colitis.[5]

Lee Simmons and co-workers first suggested that subclinical colonic ischaemia was associated with absorption of a toxic factor,[16] the clinical effects produced being similar to the known effects of bacterial endotoxin. Normal colonic mucosa provides an effective barrier to pathogenic intestinal micro-organisms, but even transient ischaemia induced by aortic cross-clamping may be sufficient to allow bacterial translocation and life-threatening infection.[3] The clinical effects of Gram-negative sepsis are thought to be caused by endotoxaemia, and patients so afflicted frequently develop MSOF and death.[17] Animal experiments have confirmed absorption of endotoxins through ischaemic bowel,[16,18] and endotoxaemia has been detected in humans with inflammatory bowel disease[19] and even after colonoscopy.[20] Endotoxaemia occurring in relation to low sigmoid $pH_i$ during and after aortic aneurysm repair has also been recently reported, with low $pH_i$ correlating with high peak systemic endotoxin and tumour necrosis factor (TNF) levels, though not interleukin-6 (IL-6).[21]

Mean sigmoid colonic $pH_i$ was close to normal before cross-clamp application, but had fallen significantly immediately prior to clamp removal (Fig. 4), though there was no direct association between clamp time and degree of ischaemia. It is most interesting to note that after clamp release, low $pH_i$ and therefore

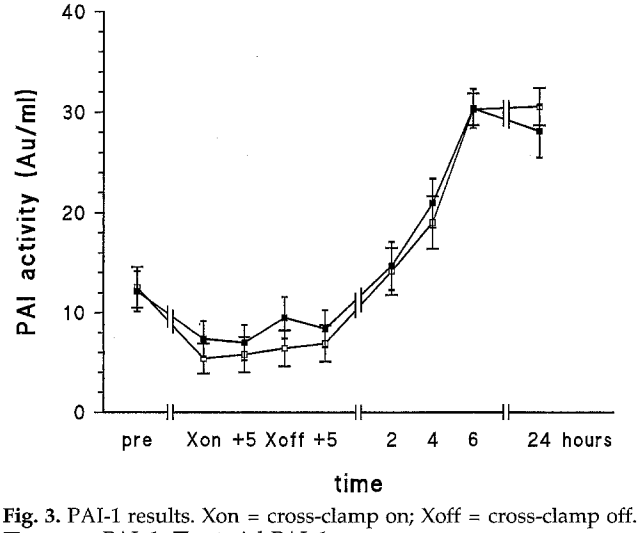


**Fig. 3.** PAI-1 results. Xon = cross-clamp on; Xoff = cross-clamp off. □ venous PAI-1; ■ arterial PAI-1.

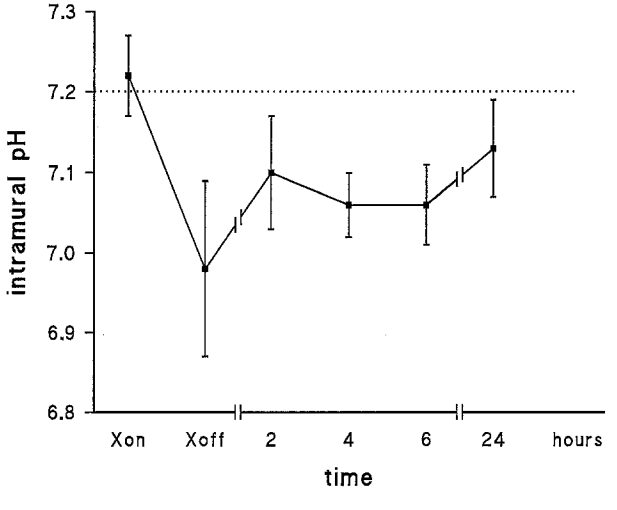


**Fig. 4.** Tonometry results. .... = normal value for $pH_i$; Xon = cross-clamp on; Xoff = cross-clamp off.

ischaemia persisted in 53% of patients at 6 hours; by 24 hours $pH_i$ remained low in 32%. Peak levels of endotoxaemia were seen immediately prior to cross-clamp removal (Fig. 2), corresponding with the period of maximum colonic ischaemia, and probably occurs due to increased colonic mucosal permeability secondary to clamp-induced ischaemia. The second lesser peak at 6 hours may result in part from persistent colonic ischaemia and also from reperfusion injury due to free radical activation. In this study, those patients with high endotoxin levels during the cross-clamp period had a significantly higher morbidity and mortality, with two fatal myocardial infarctions and two patients developing overt renal impairment; all four patients had endotoxaemia with values greater than 100 pg/ml prior to clamp removal. At 24 hours endotoxin levels had returned to pre-operative values.

Activation of the fibrinolytic system has also been clearly demonstrated in this study, represented by extremely high levels of PAI-1 activity (Fig. 3), a reliable indicator of fibrinolytic activation. Significantly elevated PAI-1 levels had occurred by 6 hours postoperatively, and were still present at 24 hours although peak levels had passed. There was no correlation between peak endotoxin levels during the crossclamp phase, and the rate of subsequent PAI-1 elevation. PAI-1 is one of the cytokines released as part of the acute phase reaction, and in this study endothelial damage either as a direct result of graft insertion or due to lower limb ischaemia induced by cross-clamping may also have contributed. Injection of endotoxin in rabbits has induced increased PAI-1 activity;[22] though this may be direct or indirect via induction of TNF and/or interleukin-1.[23] The latter two compounds, as well as endotoxin, stimulate PAI-1 release and down-regulate tPA release by endothelial cells.[24,25] The occurrence of virtually identical values in both venous and arterial blood indicated that such responses were systemic and not due to local trauma of venepuncture. Gough *et al.*[26] found that basal arterial PAI-1 activity was significantly greater than venous PAI-1 activity, but this study has shown that this differential is abolished, possibly due to the significant vascular insult involved in aortic cross-clamping, and that PAI-1 activity throughout the circulation remains depressed for over an hour post-operatively before a generalised elevation at 6 hours, lasting to 24 hours at least.

This study has highlighted several important issues. Colonic ischaemia, transient and subclinical in most instances, is much more common than previously thought, and is associated with endotoxaemia and PAI-1 release. Sigmoid tonometry is a simple, safe and accurate means of monitoring colonic intramural pH, and persistent ischaemia may prompt early colonoscopy or prophylactic treatment with antibodies to endotoxin to reduce systemic complications. Whilst the current COATEST endotoxin assay is sensitive, its methodology and expense precludes its use for individual sample measurement. The development of a more economical and robust endotoxin assay would obviously have important therapeutic implications. The rôle of fibrinolysis, in particular the release of PAI-1, in the pathophysiology of septic shock is still not well established.[27] As well as increasing PAI-1, septicaemia may also be thrombogenic via exposure of tissue factor on endothelial cells.[28] Under physiological conditions intravascular fibrin deposits are removed by the action of the fibrinolytic system; in septicaemia and septic shock this protective mechanism may be compromised by the induction of PAI-1. Whilst the significance of PAI-1 elevation remains uncertain, nonetheless it has been reported as a strong predictor of mortality due to septicaemia,[2] the results in this study do not fully support this hypothesis, and though endotoxaemia precedes PAI-1 release, it is likely that the latter is multifactorial as no direct causal relationship could be found.

In conclusion, perioperative endotoxaemia appears to be an important prognostic indicator of morbidity following aortic surgery. Such a role is not necessarily played by fibrinolytic activation, though this is associated with the endotoxic response. Subclinical colonic ischaemia is probably the most important factor predisposing to endotoxaemia; its early detection using the sigmoid tonometer may allow identification of patients at greatest risk.

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